

dCas9-NLS-3xHA-VP64

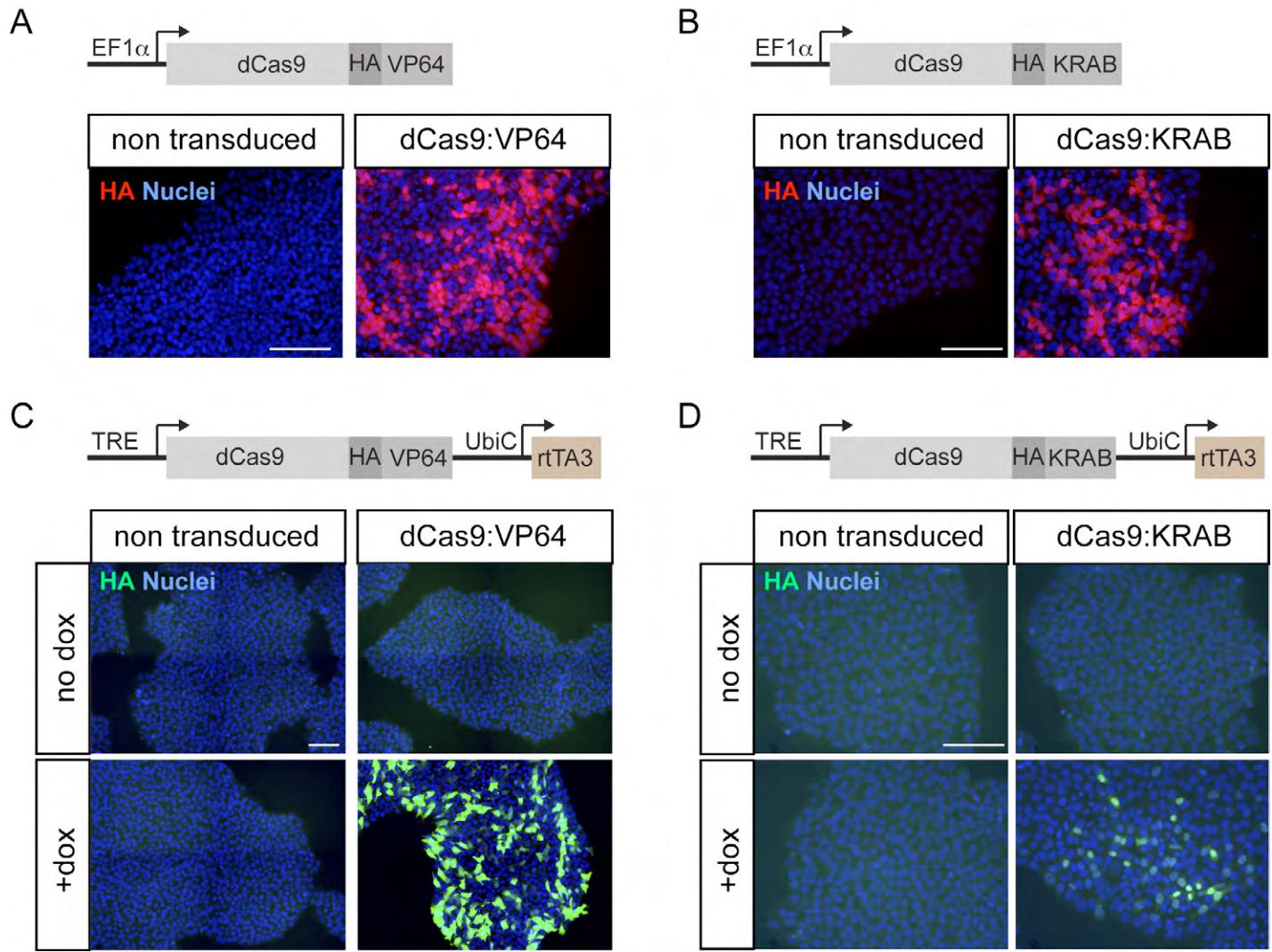
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DFDLDMLIN*

dCas9-NLS-3xHA-KRAB

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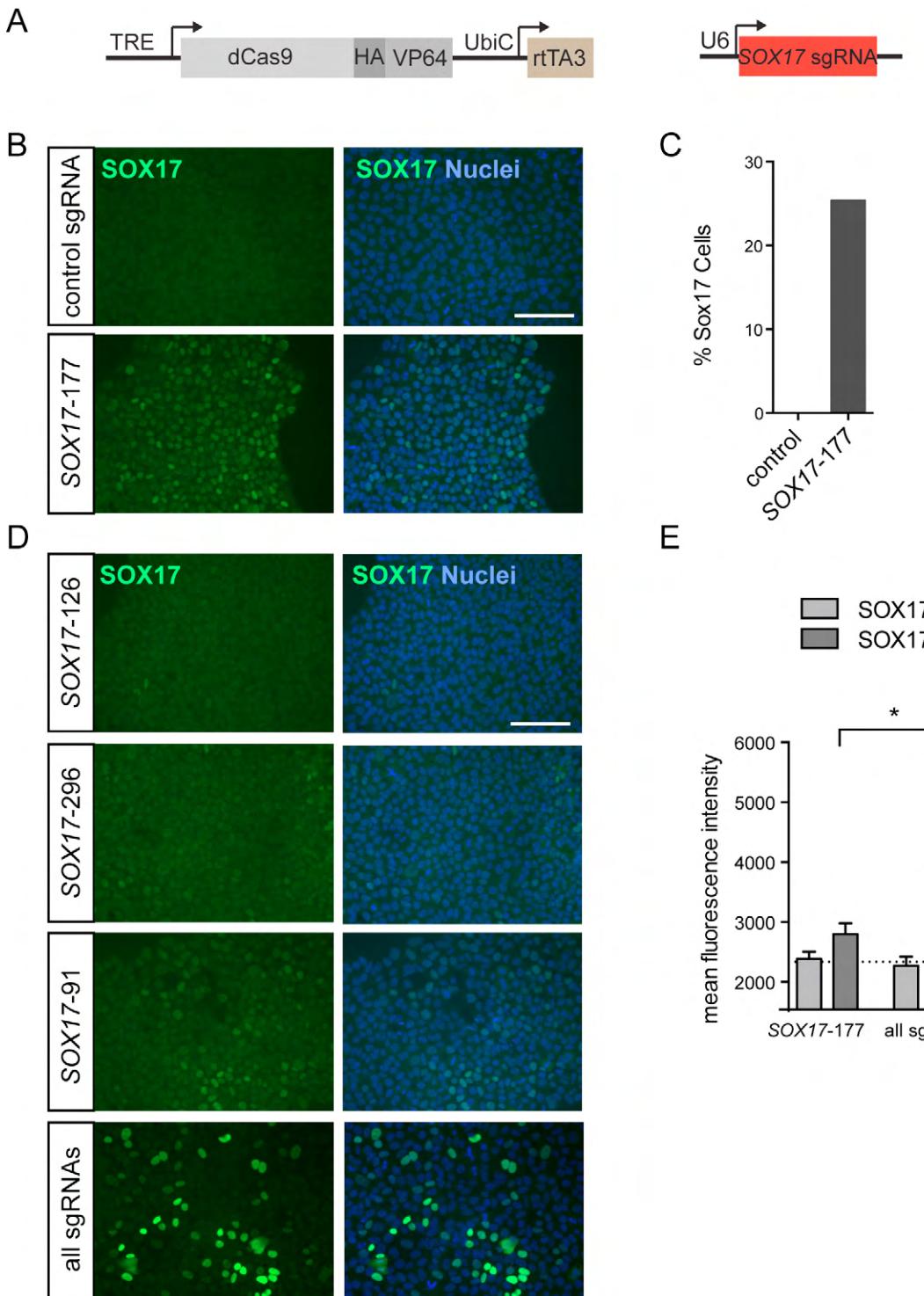
Supplementary Figure S1: dCas9-E amino acid sequences and sgRNA cloning template

Amino acid sequences of dCas9-NLS-3xHA-VP64 and dCas9-NLS-3xHA-KRAB, where the D10A, H840A spCas9 sequence is indicated in yellow, the SV40 NLS in cyan, the 3xHA tag in green and the effector domain in magenta. The pLKO.1 U6 promoter sgRNA template DNA sequence is shown where the U6 promoter transcription start site is indicated in magenta and the constant portion of the sgRNA sequence is highlighted in yellow (Mali et al., 2013). The type IIS BfuAI recognition sites utilized for cloning annealed oligonucleotides encoding the sgRNA sequence are indicated in bold letters, where the cleavage positions on the Crick and Watson strands are respectively indicated above and below the sequence yielding 4 base pair 5' overhangs.



Supplementary Figure S2: dCas9-E variants can be expressed in hESCs

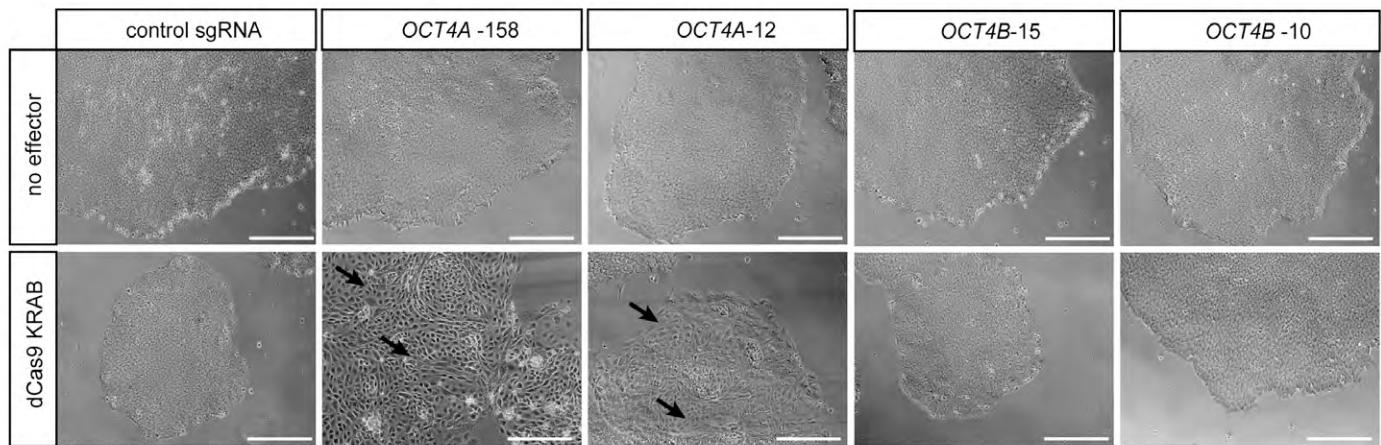
Delivery of dCas9-E variants is detected by immunofluorescence through an internal HA-tag. (A) Immunofluorescence analysis for HA in hESCs transduced with EF1 α -regulated dCas9-VP64. (B) Immunofluorescence analysis for HA in hESCs transduced with EF1 α -regulated dCas9-KRAB. (C) Immunofluorescence analysis for HA in hESCs transduced with TRE-regulated dCas9-VP64 after 48 hours with and without doxycycline treatment. (D) Immunofluorescence analysis for HA in hESCs transduced with TRE-regulated dCas9-KRAB after 48 hours with and without doxycycline treatment. Scale bars = 100 μ m.



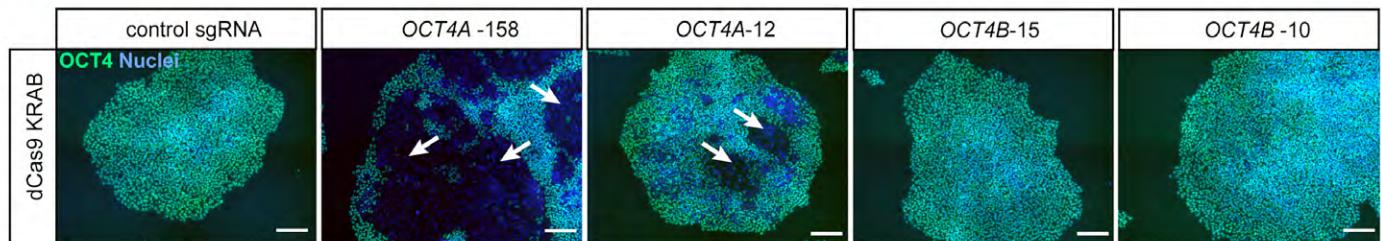
Supplementary Figure S3: Quantitation of CRISPRa-mediated, sgRNA-specific upregulation of a developmentally relevant transcription factor in hESCs

(A) Schematic of the inducible TRE-regulated dCas9-VP64 and *SOX17* sgRNA construct. (B) Immunofluorescence analysis of TRE-regulated dCas9-VP64 cells transduced with a control or *SOX17*-177 sgRNA after 6 days of doxycycline treatment. (C) Percentage of cells expressing *SOX17* in TRE-regulated dCas9-VP64 cells transduced with a control or *SOX17*-177 sgRNA after 6 days of doxycycline treatment. (D) Immunofluorescence analysis of TRE-regulated dCas9-VP64 cells transduced with *SOX17*-126, *SOX17*-296 or *SOX17*-91 sgRNA, or a combination of all four *SOX17* sgRNAs after 6 days of doxycycline treatment. (E) Mean fluorescence intensity of *SOX17*- and *SOX17*+ populations in TRE-regulated dCas9-VP64 cell cultures transduced with *SOX17*-177 sgRNA or a combination of four *SOX17* sgRNAs after 6 days of doxycycline treatment. The background mean fluorescence intensity of control sgRNA cultures is represented with a dotted line. The difference in mean fluorescence intensity of *SOX17*+ populations between single and multiple sgRNAs is statistically significant at $p < 0.01$ using a Mann-Whitney test. Scale bars = 100 μ m.

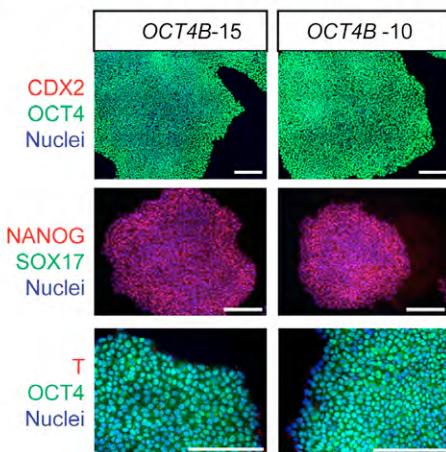
A



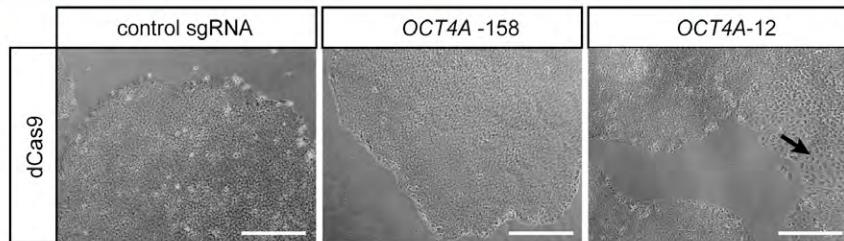
B



C



D



Supplementary Figure S4: CRISPRi-mediated downregulation of *OCT4A* in hESCs induces morphological changes and differentiation

(A) Phase contrast images of cells expressing control, *OCT4A* or *OCT4B* sgRNAs in control or TRE-regulated dCas9-KRAB cells after 6 days of doxycycline treatment. Morphological changes indicated by arrows. (B) *OCT4A* immunofluorescence analysis of cells expressing control, *OCT4A* or *OCT4B* sgRNAs in TRE-regulated dCas9-KRAB cells after 6 days of doxycycline treatment. Areas of cells not expressing *OCT4A* indicated by arrows. (C) Immunofluorescence analysis of TRE-regulated dCas9-KRAB cells expressing *OCT4B* sgRNAs after 6 days of doxycycline treatment for CDX2, *OCT4A*, NANOG, SOX17 and T. (D) Phase contrast images of cells expressing *OCT4A* or unrelated sgRNA in TRE-regulated dCas9 cells after 6 days of doxycycline treatment. Morphological changes indicated by arrows. Scale bars = 200 μ m.

SUPPLEMENTARY MATERIAL

SUPPLEMENTARY TABLES

Supplementary Table 1: sgRNAs

Name	Target Promoter	Position to TSS	Target Strand	Target Sequence (including PAM)
OCT4A	oct4 isoform A	-158	template	GGGGCGCCAGTTGTCTCCGG
OCT4A	oct4 isoform A	-12	template	GTGGGACTGGGGAGGGAGAGAGG
OCT4B	oct4 isoform B	-10	coding	GGGTCCCACAAACTATAACATGG
OCT4B	oct4 isoform B	-15	template	GCATGCCATGTTATAGTTGTGG
SOX17	sox17	-126	template	GGAGGGGCAAGGGCGGGCGTGG
SOX17	sox17	-177	template	GCTCCGGCTAGTTCCGGGG
SOX17	sox17	-296	template	GGGCAAGTACGTCGATTCCAAGG
SOX17	sox17	-91	template	GGGCGTGGCCTAACGACGCGGG
CAG	CAG		template	GTTCCCGCGTTACATAACTTACGG

Supplementary Table 2: Primary antibodies

Antibody	Source	Dilution
CDX2	Mouse monoclonal, BioGenex MU392A-UC	1:300
HA	Rat monoclonal, Roche 11867431001	1:500
NANOG	Rabbit polyclonal, Abcam ab21624	1:400
OCT4A	Mouse monoclonal, Santa Cruz Biotechnology sc-5279	1:100
OCT4A	Goat polyclonal, Santa Cruz Biotechnology sc-8628	1:500
SOX17	Goat polyclonal, R&D Systems AF1924	1:300
T	Goat polyclonal, Santa Cruz Biotechnology sc-17743	1:300

Supplementary Table 3: qPCR primers

Gene	Forward Primer	Reverse Primer
ACTB	TGGCACCAACACCTTCTACAATGA	CAGCCTGGATAGCAACGTACAT
AFP	AGAACCTGTCACAAGCTGTG	GACAGCAAGCTGAGGATGTC
CDX2	GGGCTCTCTGAGAGGCAGGT	CCTTGCTCTGCGGTTCTG
SOX7	ACGCCGAGCTCAGCAAGAT	TCCACGTACGGCCTCTTCTG
T	TGCTTCCCTGAGACCCAGTT	GATCACTTCTTCCTTGCATCAAG